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A Single Dominant Gene Controlling Resistance to Soil Zinc Deficiency in Common Bean

Shree P. Singh* and Dale T. Westermann

ABSTRACT

Cultivated soils often are either deficient or possess toxic concentrations of one or more mineral elements that adversely affect emergence, growth, maturity, production potential, and/or nutritional quality of common bean (*Phaseolus vulgaris* L.). Our objective was to study the inheritance of resistance to soil Zn deficiency. The resistant 'Matterhorn' was crossed with the susceptible 'T-39'. The F_1 was backcrossed to Matterhorn (BC_1) and T-39 (BC_2), and advanced to the F_2. The two parents, F_1, F_2, BC_1, and BC_2 were evaluated in a Zn deficient field trial at Kimberly, Idaho in 2001. Plants were classified as tall-healthy or stunted with chlorotic leaves. Leaves were sampled from the two types of plants at flowering and analyzed for Zn concentration. The tall plants had an average leaf Zn concentration of 22.5 mg kg^{-1}. In contrast, stunted plants had a Zn concentration of 15.0 mg kg^{-1}. All F_1 plants were tall resembling Matterhorn, except that unlike Matterhorn (white flowers and seeds) they had purple flowers and black shiny seeds. Thus, the resistance to Zn deficiency was dominant. A segregation of 45 resistant (R) to 20 susceptible (S) plants was observed in the F_2, giving a good fit to 3 R:1 S ($\chi^2 = 1.1538$, $P = 0.28$). All plants in BC_1 were resistant. In BC_2, 142 R and 139 S plants were observed, giving a ratio of 1 R to 1 S ($\chi^2 = 0.032$, $P = 0.86$). This supports a single dominant gene controlling soil Zn deficiency resistance. The symbol *Znd* is proposed for the dominant allele controlling resistance to soil Zn deficiency, and *znd* for its susceptible counterpart.

As much as 60% of the soils in the common bean production regions around the world suffer from mineral deficiencies or toxicities (CIAT, 1992; Thung and Rao, 1999; Wortmann et al., 1998). The calcareous soils of northwestern USA generally have excess soluble salts such as Ca, K, Mg, and Na. This increases soil pH and may cause Zn, P, Fe, and/or Mn deficiencies. Brown and Leggett (1967) and Leggett et al. (1975) reported a widespread Zn deficiency in the common bean crop in the Magic Valley of southern Idaho. Zinc deficiency was also reported in common bean production regions in Michigan (Judy et al., 1965; Polson and Adams, 1970) and North Dakota (Moraghan and Grafton, 1999). LeBaron (1966) reported that a preceding crop of sugarbeet (*Beta vulgaris* L.), high manure use, or phosphate fertilizers can intensify Zn deficiency symptoms on susceptible common bean cultivars. Similarly, land leveling or deep plowing that brings the highly calcareous subsoil to the surface may also enhance Zn deficiency (Brown and Leggett, 1967). LeBaron (1966) and LeBaron et al. (1971) suggested applying 11 kg Zn ha^{-1} would correct problem soils for common bean production.

Blaylock (1995), Boawn et al. (1969), and Brown and Leggett (1967) described the visual symptoms of Zn deficiency and showed that common bean cultivars vary in sensitivity to Zn deficiency. Shortening of internodes or plant stunting, interveinal chlorosis and bronzing of leaves, delayed flowering and maturity, and reduced biomass production and seed yield are common symptoms. Edwards and Mohamed (1973) also reported a reduction in carbonic anhydrase activity in leaves of Zn deficient common bean cultivars. Yield losses of susceptible small-seeded (<25 g 100 seed-weight^{-1}) common bean cultivars (e.g., Mackinac, Sanilac, Seaway, and T-39) in moderately Zn deficient soils could reach up to 100% (Brown and Leggett, 1967; Westermann and Singh, 2000). Zinc uptake and its concentration in plant parts and seed are lower in sensitive or inefficient cultivars compared with tolerant or efficient cultivars (Brown and Leggett, 1967; Moraghan and Grafton, 1999). Brouwer et al. (1981) and Polson and Adams (1970) observed differential cultivar response to Zn foliar sprays in navy beans. Moreover, even the most resistant snap and dry common bean cultivars responded positively to Zn application as evidenced by their increased dry weight and Zn concentration (Brown and Leggett, 1967).

Soil Zn deficiency resistant common bean cultivars must employ a plethora of physiological mechanisms to tolerate Zn deficiency stress better than their susceptible counterparts. These supposedly Zn-efficient genotypes should have greater fertilizer efficiency and a greater harvest index compared to Zn-inefficient or susceptible genotypes when grown in low Zn soils. The increased fertilizer efficiency of the Zn-deficiency resistant common bean genotypes offers a potential to manage severely Zn-deficient soils by a combination of growing Zn-deficiency resistant cultivars and Zn fertilization at low rates. Nonetheless, managing soil Zn-deficiency stress through fertilizer use increases production costs and reduces the competitive edge of common bean growers in global markets.

Westermann and Singh (2000) found a positive correlation between seed yield and Zn concentration in leaves of 36 common bean genotypes grown in Zn-deficient soil at Kimberly, ID. There were marked differences in leaf yellowing–bronzing (scores 2–9, where 1 = symp-

S.P. Singh, Univ. of Idaho, 3793 N 3600 E, Kimberly, ID 83341-5076; D.T. Westermann, USDA-ARS Northwest Irrigation & Soils Research Laboratory, 3793 N 3600 E, Kimberly, ID 83341-5076. Published as Idaho Agric. Exp. Stn. J. Article No. 02703, Univ. of Idaho, College of Agriculture & Life Sciences, Moscow, ID 83844. Received 18 Sep. 2001. *Corresponding author (singh@kimberly.uidaho.edu).

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e 1. Soil chemical characteristics and available nutrient con-ntration of a calcareous Portneuf silt loam soil in 1999 at imberly, ID, used for screening common bean genotypes from 99 to 2001.

:haracteristic	Range	Critical level for common bean
nic carbon (mg g^{-1})	4–6	–
(cmol$_c$ kg^{-1})	18.0–20.0	–
	7.8–8.2	–
iesium (cmol$_c$ kg^{-1})	4.0–6.0	–
ssium (cmol$_c$ kg^{-1})	0.4–0.6	<0.13
(mg kg^{-1})	0.6–0.8	<1.0
(mg kg^{-1})	12.0–13.0	<4.5
ganese (mg kg^{-1})	9.6–9.9	<1.0
phorus (mg kg^{-1})	10.0–12.0	<8.0

lless and 9 = severe yellowing and bronzing), plant ıting, and seed yield (11–4722 kg ha^{-1}). The small-ded navy and black market classes of common bean, general, were more susceptible to Zn deficiency. In trast, the highest resistance was observed in medium-ded (25–40 g 100 seed-weight^{-1}) great northern, k, pinto, and red market classes, followed by large-ded (>40 g 100 seed-weight^{-1}) common beans. Our ective was to study the inheritance of resistance to deficiency in common bean.

MATERIALS AND METHODS

Zinc-deficiency resistant Matterhorn was crossed with sus-tible common bean cultivar T-39 in 2000. Both were evalu-l in a Zn-deficient soil (Table 1) at Kimberly, ID, from 9 to 2001 (1190 m elevation; 23.5°C average maximum air perature from April to September). Soil was a Portneuf loam (coarse, mixed, superactive, mesic Durinodic Xeric ocalids) with approximately 1% slope. Composite soil ples were taken prior to planting from each replication analyzed for soil chemical characteristics and available ients (Table 1). In all three years, the response of both ivars was similar. Matterhorn was highly resistant, receiv-a score of 2 on a 1 to 9 score, where 1 = tall healthy plants 1 no visible Zn-deficiency symptoms, and 9 = severe leaf orosis and bronzing, plant stunting, and considerable seed-death. T-39 had scores ranging from 7 to 9. Matterhorn white medium-seeded, high yielding, widely adapted great thern cultivar developed at Michigan State University lly et al., 1999). Matterhorn has an indeterminate upright wth habit (Type II) with small vine, and the *I* gene for stance to *Bean common mosaic virus* (BCMV). Matter-n is also resistant to most common races of the fungus *myces appendiculatus* (Pers.:Pers.) Unger, causing bean in the USA. T-39 is a small-seeded black bean cultivar cted from the old 'Black Turtle Soup' landrace. T-39 was ased jointly by the University of California-Davis and Cor-University, Ithaca, NY, in 1974. Like Matterhorn, T-39 has indeterminate upright growth habit (Type II) with ll vine and *I* gene resistance to BCMV. It was also report-o be resistant to rust and susceptible to common bacter-blight [caused by *Xanthomonas campestris* pv. *phaseoli* ith) Dye], white mold [caused by *Sclerotinia sclerotiorum*) de Bary], and Alpha, Beta, and Delta races of *Colletotri-m lindemuthianum* (Sacc. & Magn.) Bri. & Cav. causing hracnose at the time of release in the USA.

The Matterhorn/T-39 F_1 hybrid was backcrossed to Matter-n (BC_1) and T-39 (BC_2), and also allowed to produce F_2 d. Matterhorn, T-39, and their F_1, F_2, BC_1, and BC_2 were luated in a marginally Zn-deficient soil (the same field used in 1999 and 2000) in 2001. A randomized complete block design with three replicates was used. Each plot consisted of a single row, 6 m long, spaced 0.56 m apart. Within row plant spacing was approximately 70 mm. An average of 30 seeds plot^{-1} were planted for each parent, 15 seeds for F_1, 25 seeds for F_2 and BC_1, and 100 seeds for BC_2. Hand weeding and herbicides were used to keep plots free from weeds during the growing season. Gravity irrigation was used to apply Snake River water as necessary to assure optimum crop growth and development. However, fertilizer was not applied to any plots.

Total plant counts were made in each plot within the first week after emergence. Visual Zn-deficiency symptoms, if any, were also scored on a 1-to-9 scale as explained above, beginning 3 wk after emergence. Plants within each plot were classified into tall healthy with very mild or no visual Zn deficiency (receiving scores of ≤3) and short or stunted with severe foliar Zn deficiencies (receiving scores of ≥7). No plants in any plot received a score of 4, 5, or 6. One most recently matured trifoliolate leaf was excised from each tall resistant and stunted susceptible plant in each plot and in each replicate at approximately one-tenth bloom. The petiole was discarded and leaflets dried at 60°C, ground, and analyzed by ICP-OES for Zn concentrations after dry ashing at 500°C and solublizing the ash in nitric acid (Gavlak et al., 1998). The observed frequencies of resistant and susceptible plants in segregating genotypes were compared with expected frequencies by a χ^2 test.

RESULTS AND DISCUSSION

The tip and borders of primary leaves of 10- to 14-d-old seedlings of T-39 began to show yellowing. Severe interveinal chlorosis and bronzing of primary and subsequent trifoliolate leaves, shortening of internodes, and plant stunting followed. Some plants also began to die 3 wk after planting (received a score of 9). The surviving plants did not flower, produce seed, or reach maturity in 100 d (received a score of 7 or 8). The average height at 90 d was 200 mm. In contrast, no visible symptoms of Zn deficiency were observed in leaves of seedlings or adult plants of Matterhorn. Its mean plant height was 60.0 cm and it flowered and produced seed as expected for nonZn-deficient soils at Kimberly, ID.

The seedlings as well as adult plants of the Matterhorn/T-39 F_1 were as normal and healthy as Matterhorn. However, Matterhorn had white flowers and seeds. All F_1 plants had purple flowers and black shiny seeds. Thus, resistance to Zn deficiency was a dominant trait and not linked to flower or seed color.

In the F_2, both the stunted plants with typical Zn-deficiency symptoms similar to T-39 (susceptible) and healthy tall plants like Matterhorn (resistant) were observed. There were 45 Zn-deficiency resistant and 20 susceptible plants (Table 2). These observed frequencies gave a good fit to the expected 3 resistant to 1 susceptible ratio (χ^2 = 1.1538, P = 0.28). Thus, a monogenic dominant inheritance of resistance to Zn deficiency in common bean was indicated.

All plants in the BC_1 (the F_1 backcrossed to Matterhorn) were tall and healthy like Matterhorn (Table 1). However, they segregated for purple and white flower and white, black and other seed colors, as would be expected because both white flower and seed colors are recessive traits (Prakken, 1970; Smith, 1961). Since all

Table 2. Mean leaf zinc (Zn) concentration, observed frequencies, expected ratio, and χ^2 values for common bean cultivars Matterhorn, T-39, and their F_1, F_2, and backcross generations evaluated in Zn deficient soil at Kimberly, Idaho in 2001.

Genotype	Leaf Zn concentration	Observed frequencies		Expected ratio	χ^2 value	*P*
		Tall	Stunted			
	mg kg^{-1}	no	no			
Matterhorn	24.8	78		-	-	
T-39	13.9	-	65		-	-
Matterhorn/T-39 (F_1)	21.8	32	-		-	-
Matterhorn/T-39 (F_2)	24.7 tall: 19.6 stunted	45	20	49 tall: 16 stunted	1.1538	0.28
Matterhorn/Matterhorn/T-39 (BC_1)	23.3	71	-		-	-
T-39//Matterhorn/T-39 (BC_2)	17.8 tall: 11.6 stunted	142	139	140 tall: 140 stunted	0.032	0.86

plants of the Matterhorn/T-39 F_1 were normal and healthy as Matterhorn and because a monogenic dominant inheritance of resistance to Zn deficiency was observed in the F_2, only normal and healthy plants would be expected in the BC_1.

The F_1 backcrossed to T-39 (BC_2) had 142 healthy tall and 139 Zn deficient stunted plants (Table 2). This gave a good fit to the expected resistant to susceptible ratio of 1:1 ($\chi^2 = 0.032$, $P = 0.86$). Thus, once again the results observed were consistent with those in F_1, F_2, and BC_1, where a single dominant gene controlled resistance to Zn deficiency in common bean. We propose using the symbol *Znd* for the dominant allele controlling resistance to Zn deficiency in high pH calcareous soils, and *znd* for its susceptible counterpart. Recently, Forster et al. (2002) also reported a single dominant gene that controlled seed-Zn accumulation in the efficient navy bean cultivar Voyager when crossed to the inefficient 'Albion' navy bean, and progenies evaluated in Zn-deficient soil in North Dakota. It is not known if Matterhorn and Voyager carry the same or different genes.

Unlike quantitative inheritance with low to intermediate heritability for P absorption, P utilization, and low soil P tolerance (Lindgren et al., 1977; Fawole et al., 1982; Urrea and Singh, 1989), the monogenic dominant control of Zn-deficiency resistance in common bean should facilitate and expedite its transfer into susceptible cultivars such as T-39, Sanilac, and Mackinac. Alternative backcross, pedigree, single-seed-descent, or gamete selection methods could be used depending upon the genetic distance between parental genotypes, other objectives of the program, urgency of the project, and available resources. Also, molecular markers tightly linked to the resistant and/or susceptible allele could be identified. This should facilitate both transfer of Zn-deficiency resistance into susceptible genotypes and fingerprinting of cultivars. Thus, growers could choose either to plant only resistant cultivars or preplant apply adequate quantities of Zn fertilizer in problem soils.

Because large-seeded common bean cultivars, in general, also have high levels of resistance (Westermann and Singh, 2000) and are of distinct evolutionary origin (Gepts and Bliss, 1985; Becerra-Velsquez and Gepts, 1994; Singh et al., 1991), it would be useful to know if the same or different gene(s) control resistance to Zn deficiency in them. Should the genetic control in large-seeded Andean and small-seeded Middle American common bean be different from and complementary to that found in medium-seeded Middle American common bean, combining them for increased levels of resistance would be worthwhile.

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